

Claims

1. A method for selecting siRNA comprising selecting an siRNA molecule of 19 – 25 nucleoside bases, said method comprising:

(a) selecting a target gene;

- 5 (b) measuring the functionality of sequences of 19 – 25 nucleotides in length that are substantially complementary to a stretch of nucleotides of the target sequence, wherein said functionality is dependent upon non-target specific criteria.

- 10 2. The method according to claim 1 wherein said functionality is determined by applying one of the following formulas:

Formula I = $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

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Formula II = $-(GC/3) - (AU_{15-19}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3)$;

Formula III = $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C}) * 3$;

20

Formula IV = $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C}) * 2 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula V = $-(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

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Formula VI = $-(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3)$;

Formula VII = $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C}) * 1 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 3 + (A_3) * 3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2$;

30

wherein in Formulas I – VII:

$AU_{15-19} = 0 - 5$ depending on the number of A or U bases on the sense

strand at positions 15 –19;

$G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;

$C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

$Tm_{20^{\circ}C} = 1$ if the Tm is greater than $20^{\circ}C$;

$A_3 = 1$ if A is the base at position 3 on the sense strand, otherwise its value is 0;

$A_{11} = 1$ if A is the base at position 11 on the sense strand, otherwise its value is 0;

$A_{14} = 1$ if A is the base at position 14 on the sense strand, otherwise its value is 0;

$A_{19} = 1$ if A is the base at position 19 on the sense strand, otherwise its value is 0;

$U_5 = 1$ if U is the base at position 5 on the sense strand, otherwise its value is 0;

$U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;

or,

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein

$A_1 = 1$ if A is the base at position 1 of the sense strand, otherwise its value is 0;
 $A_2 = 1$ if A is the base at position 2 of the sense strand, otherwise its value is 0;
 $A_3 = 1$ if A is the base at position 3 of the sense strand, otherwise its value is 0;
 $A_4 = 1$ if A is the base at position 4 of the sense strand, otherwise its value is 0;
5 $A_5 = 1$ if A is the base at position 5 of the sense strand, otherwise its value is 0;
 $A_6 = 1$ if A is the base at position 6 of the sense strand, otherwise its value is 0;
 $A_7 = 1$ if A is the base at position 7 of the sense strand, otherwise its value is 0;
 $A_{10} = 1$ if A is the base at position 10 of the sense strand, otherwise its value is 0;
 $A_{11} = 1$ if A is the base at position 11 of the sense strand, otherwise its value is 0;
10 $A_{13} = 1$ if A is the base at position 13 of the sense strand, otherwise its value is 0;
 $A_{19} = 1$ if A is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

$C_3 = 1$ if C is the base at position 3 of the sense strand, otherwise its value is 0;
15 $C_4 = 1$ if C is the base at position 4 of the sense strand, otherwise its value is 0;
 $C_5 = 1$ if C is the base at position 5 of the sense strand, otherwise its value is 0;
 $C_6 = 1$ if C is the base at position 6 of the sense strand, otherwise its value is 0;
 $C_7 = 1$ if C is the base at position 7 of the sense strand, otherwise its value is 0;
 $C_9 = 1$ if C is the base at position 9 of the sense strand, otherwise its value is 0;
20 $C_{17} = 1$ if C is the base at position 17 of the sense strand, otherwise its value is 0;
 $C_{18} = 1$ if C is the base at position 18 of the sense strand, otherwise its value is 0;
 $C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

25 $G_1 = 1$ if G is the base at position 1 on the sense strand, otherwise its value is 0;
 $G_2 = 1$ if G is the base at position 2 of the sense strand, otherwise its value is 0;
 $G_8 = 1$ if G is the base at position 8 on the sense strand, otherwise its value is 0;
 $G_{10} = 1$ if G is the base at position 10 on the sense strand, otherwise its value is 0;
 $G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;
30 $G_{19} = 1$ if G is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

$U_1 = 1$ if U is the base at position 1 on the sense strand, otherwise its value is 0;
 $U_2 = 1$ if U is the base at position 2 on the sense strand, otherwise its value is 0;

$U_3 = 1$ if U is the base at position 3 on the sense strand, otherwise its value is 0;
 $U_4 = 1$ if U is the base at position 4 on the sense strand, otherwise its value is 0;
 $U_7 = 1$ if U is the base at position 7 on the sense strand, otherwise its value is 0;
 $U_9 = 1$ if U is the base at position 9 on the sense strand, otherwise its value is 0;
5 $U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;
 $U_{15} = 1$ if U is the base at position 15 on the sense strand, otherwise its value is 0;
 $U_{16} = 1$ if U is the base at position 16 on the sense strand, otherwise its value is 0;
 $U_{17} = 1$ if U is the base at position 17 on the sense strand, otherwise its value is 0;
 $U_{18} = 1$ if U is the base at position 18 on the sense strand, otherwise its value is 0;

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GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand
or within positions 15 – 18 if the sense strand is only 18 base pairs in length;

GC_{total} = the number of G and C bases in the sense strand;

15 $T_m = 100$ if the targeting site contains an inverted repeat longer than 4 base pairs,
otherwise its value is 0; and

X = the number of times that the same nucleotide repeats four or more times in a
row.

20 3. A method of gene-silencing comprising selecting an siRNA according to
claim 2 and introducing it into a cell.

4. The method according to claim 3 wherein said introducing is by allowing
passive uptake of the siRNA.

25 5. The method according to claim 3, wherein said introducing is through the
use of a vector.

30 6. A method for developing an siRNA algorithm for selecting siRNA, said
method comprising:
(a) selecting a set of siRNA;
(b) measuring the gene silencing ability of each siRNA from said set;
(c) determining the relative functionality of each siRNA;
(d) determining the amount of improved functionality by the presence or
absence of at least one variable selected from the group consisting of

- the total GC content, melting temperature of the siRNA, GC content at positions 15 –19, the presence or absence of a particular nucleotide at a particular position and the number of times that the same nucleotide repeats within a given sequence; and
- 5 (e) developing an algorithm using the information of step (d).
7. A method of selecting an siRNA with improved functionality, said method comprising using the algorithm of claim 6.
- 10 8. A method of selecting hyperfunctional siRNA, said method comprising using at least one functional siRNA, wherein at least one said functional siRNA has been selected according to the method of claim 7 and measuring the silencing ability of said at least one functional siRNA, wherein silencing ability is measured at a concentration of less than 1
- 15 nanomolar siRNA.
9. An siRNA molecule, wherein said siRNA molecule is effective at silencing Bcl-2.
- 20 10. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a sequence substantially similar to a sequence selected from the group consisting of GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301); GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302); GUACGACAACCGGGAGUA (SEQ. ID NO. 303);
- 25 AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304); UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305); CAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306); UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307); GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);
- 30 GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).
11. The siRNA molecule of claim 10, wherein said siRNA molecule comprises a sequence selected from the group consisting of

GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301);
GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302);
GUACGACAACCGGGAGUA (SEQ. ID NO. 303);
AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304);
5 UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305);
GCAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306);
UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307);
GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);
GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and
10 GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

12. The siRNA molecule of claim 11, wherein said siRNA molecule comprises GCAUGCGGCCUCUGUUUGA .
13. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a sense strand and an anti-sense strand.
14. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a hairpin.
15. The siRNA molecule of claim 9, wherein said siRNA molecule comprises between 18 and 30 base pairs.
16. A kit for gene silencing comprising at least one siRNA selected from the group consisting of sequences substantially similar to the group consisting of GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301);
GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302);
GUACGACAACCGGGAGUA (SEQ. ID NO. 303);
AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304);
25 UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305);
GCAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306);
UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307);
30 GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);

GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and
GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

- 5 17. A method of gene silencing comprising using the siRNA molecule of claim 10.
18. A method of gene silencing comprising using the siRNA molecule of claim 11.
- 10 19. A kit, wherein said kit is comprised of at least two siRNA, wherein said at least two siRNA comprise a first optimized siRNA and a second optimized siRNA, wherein said first optimized siRNA and said second optimized siRNA are optimized according to one of the following formulas:
- 15 Formula I = $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;
- Formula II = $-(GC/3) - (AU_{15-19}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3)$;
- 20 Formula III = $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C}) * 3$;
- Formula IV = $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C}) * 2 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;
- 25 Formula V = $-(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;
- Formula VI = $-(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3)$;
- Formula VII = $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C}) * 1 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 3 + (A_3) * 3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2$;
- 30

wherein in Formulas I – VII:

$AU_{15-19} = 0 - 5$ depending on the number of A or U bases on the sense strand at positions 15 –19;

$G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;

$C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

$Tm_{20^{\circ}C} = 1$ if the Tm is greater than $20^{\circ}C$;

$A_3 = 1$ if A is the base at position 3 on the sense strand, otherwise its value is 0;

$A_{11} = 1$ if A is the base at position 11 on the sense strand, otherwise its value is 0;

$A_{14} = 1$ if A is the base at position 14 on the sense strand, otherwise its value is 0;

$A_{19} = 1$ if A is the base at position 19 on the sense strand, otherwise its value is 0;

$U_5 = 1$ if U is the base at position 5 on the sense strand, otherwise its value is 0;

$U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;

or,

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$; and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+$

$$(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$$

wherein

- 5 $A_1 = 1$ if A is the base at position 1 of the sense strand, otherwise its value is 0;
 $A_2 = 1$ if A is the base at position 2 of the sense strand, otherwise its value is 0;
 $A_3 = 1$ if A is the base at position 3 of the sense strand, otherwise its value is 0;
 $A_4 = 1$ if A is the base at position 4 of the sense strand, otherwise its value is 0;
 $A_5 = 1$ if A is the base at position 5 of the sense strand, otherwise its value is 0;
10 $A_6 = 1$ if A is the base at position 6 of the sense strand, otherwise its value is 0;
 $A_7 = 1$ if A is the base at position 7 of the sense strand, otherwise its value is 0;
 $A_{10} = 1$ if A is the base at position 10 of the sense strand, otherwise its value is 0;
 $A_{11} = 1$ if A is the base at position 11 of the sense strand, otherwise its value is 0;
 $A_{13} = 1$ if A is the base at position 13 of the sense strand, otherwise its value is 0;
15 $A_{19} = 1$ if A is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

- $C_3 = 1$ if C is the base at position 3 of the sense strand, otherwise its value is 0;
 $C_4 = 1$ if C is the base at position 4 of the sense strand, otherwise its value is 0;
20 $C_5 = 1$ if C is the base at position 5 of the sense strand, otherwise its value is 0;
 $C_6 = 1$ if C is the base at position 6 of the sense strand, otherwise its value is 0;
 $C_7 = 1$ if C is the base at position 7 of the sense strand, otherwise its value is 0;
 $C_9 = 1$ if C is the base at position 9 of the sense strand, otherwise its value is 0;
 $C_{17} = 1$ if C is the base at position 17 of the sense strand, otherwise its value is 0;
25 $C_{18} = 1$ if C is the base at position 18 of the sense strand, otherwise its value is 0;
 $C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

- $G_1 = 1$ if G is the base at position 1 on the sense strand, otherwise its value is 0;
30 $G_2 = 1$ if G is the base at position 2 of the sense strand, otherwise its value is 0;
 $G_8 = 1$ if G is the base at position 8 on the sense strand, otherwise its value is 0;
 $G_{10} = 1$ if G is the base at position 10 on the sense strand, otherwise its value is 0;
 $G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;

$G_{19} = 1$ if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$U_1 = 1$ if U is the base at position 1 on the sense strand, otherwise its value is 0;

5 $U_2 = 1$ if U is the base at position 2 on the sense strand, otherwise its value is 0;

$U_3 = 1$ if U is the base at position 3 on the sense strand, otherwise its value is 0;

$U_4 = 1$ if U is the base at position 4 on the sense strand, otherwise its value is 0;

$U_7 = 1$ if U is the base at position 7 on the sense strand, otherwise its value is 0;

$U_9 = 1$ if U is the base at position 9 on the sense strand, otherwise its value is 0;

10 $U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;

$U_{15} = 1$ if U is the base at position 15 on the sense strand, otherwise its value is 0;

$U_{16} = 1$ if U is the base at position 16 on the sense strand, otherwise its value is 0;

$U_{17} = 1$ if U is the base at position 17 on the sense strand, otherwise its value is 0;

$U_{18} = 1$ if U is the base at position 18 on the sense strand, otherwise its value is 0;

15

$GC_{15-19} =$ the number of G and C bases within positions 15 – 19 of the sense strand
or within positions 15 –18 if the sense strand is only 18 base pairs in length;

$GC_{total} =$ the number of G and C bases in the sense strand;

$T_m = 100$ if the targeting site contains an inverted repeat longer than 4 base pairs,

20 otherwise its value is 0; and

$X =$ the number of times that the same nucleotide repeats four or more times in a row.